

CLAIMS

WE CLAIM:

1. An isolated nucleic acid comprising a polynucleotide selected from the group consisting of:

(1) a first polynucleotide that encodes an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10;

(2) a second polynucleotide that is at least 80% identical to the first polynucleotide over the entire length of the first polynucleotide;

(3) a third polynucleotide that hybridizes to the first polynucleotide under stringent or moderately stringent hybridization conditions; and

(4) a fourth polynucleotide that is a complement of the first, second or third polynucleotide,

with the proviso that a nucleic acid comprising a polynucleotide that encodes a full length synaptotagmin I or II is excluded.

2. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the first polynucleotide or a complement of the first polynucleotide wherein the first polynucleotide encodes an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

3. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the first polynucleotide or a complement of the first polynucleotide wherein the first polynucleotide encodes an amino acid sequence that is at least 90% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

4. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the first polynucleotide or a complement of the first polynucleotide wherein the first

polynucleotide encodes an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

5. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the first polynucleotide or a complement of the first polynucleotide wherein the first polynucleotide encodes an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

6. A vector comprising the polynucleotide of claim 1 operably linked to a non-native promoter.

7. A vector comprising the polynucleotide of claim 5 operably linked to a non-native promoter.

8. A host cell comprising the polynucleotide of claim 1 operably linked to a non-native promoter.

9. A host cell comprising the polynucleotide of claim 5 operably linked to a non-native promoter.

10. An isolated polypeptide comprising an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10, with the proviso that a polypeptide comprising a full length synaptotagmin I or II is excluded.

11. The isolated polypeptide of claim 10, wherein the polypeptide comprises an amino acid sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID

NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

12. The isolated polypeptide of claim 10, wherein the polypeptide comprises an amino acid sequence that is at least 90% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

13. The isolated polypeptide of claim 10, wherein the polypeptide comprises an amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

14. The isolated polypeptide of claim 10, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

15. An antibody that binds specifically to an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

16. A method for reducing BoNT/B toxicity in a human or non-human animal subject comprising the step of:

administering to the subject an agent that reduces binding between BoNT/B and an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

17. The method of claim 16, wherein the subject is a human subject.

18. The method of claim 16, wherein the agent can compete for binding to BoNT/B with an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

19. The method of claim 18, wherein the agent is a polypeptide that comprises an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-79 of SEQ ID NO:2, amino acids 32-79 of SEQ ID NO:4, amino acids 33-80 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

20. The method of claim 19, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of amino acids 1-61 of SEQ ID NO:7, amino acids 1-87 of SEQ ID NO:7, amino acids 40-87 of SEQ ID NO:7, amino acids 40-267 of SEQ ID NO:7, amino acids 1-267 of SEQ ID NO:7, amino acids 1-422 of SEQ ID NO:7, amino acids 1-61 of SEQ ID NO:9, amino acids 1-87 of SEQ ID NO:9, amino acids 40-87 of SEQ ID NO:9, amino acids 40-267 of SEQ ID NO:9, amino acids 1-267 of SEQ ID NO:9, amino acids 1-422 of SEQ ID NO:9, amino acids 1-57 of SEQ ID NO:10, amino acids 1-84 of SEQ ID NO:10, amino acids 37-84 of SEQ ID NO:10, amino acids 37-264 of SEQ ID NO:10, amino acids 1-264 of SEQ ID NO:10, and amino acids 1-419 of SEQ ID NO:10.

21. The method of claim 19, wherein the agent further comprises a ganglioside.

22. The method of claim 18, wherein the polypeptide comprises an amino acid sequence selected from amino acids 40-87 of SEQ ID NO:7, amino acids 1-87 of SEQ ID NO:7, amino acids 40-267 of SEQ ID NO:7, amino acids 1-267 of SEQ ID NO:7, amino acids 40-87, amino acids 1-87 of SEQ ID NO:9, amino acids 40-267 of SEQ ID NO:9, amino acids 1-267 of SEQ ID NO:9, amino acids 37-84 of SEQ ID NO:10, amino acids 1-84 of SEQ ID NO:10, amino acids 40-264 of SEQ ID NO:10, and amino acids 1-264 of SEQ ID NO:10.

23. The method of claim 16, wherein the agent can compete with BoNT/B for binding to an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

24. The method of claim 23, wherein the agent is an antibody specific to an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

25. The method of claim 16, wherein the agent can reduce the expression of at least of one of synaptotagmin I and II in the subject.

26. The method of claim 16, wherein the agent can reduce the binding between gangliosides and an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 53-79 of SEQ ID NO:2, amino acids 53-79 of SEQ ID NO:4, amino acids 54-80 of SEQ ID NO:5, amino acids 61-87 of SEQ ID NO:7, amino acids 61-87 of SEQ ID NO:9, amino acids 58-84 of SEQ ID NO:10.

27. The method of claim 26, wherein the agent can reduce the amount of gangliosides available for binding to a ganglioside domain of at least of one of synaptotagmin I and II in the subject.

28. The method of claim 26, wherein the agent can compete with gangliosides for binding to an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 53-79 of SEQ ID NO:2, amino acids 53-79 of SEQ ID NO:4, amino acids 54-80 of SEQ ID NO:5, amino acids 61-87 of SEQ ID NO:7, amino acids 61-87 of SEQ ID NO:9, amino acids 58-84 of SEQ ID NO:10.

29. The method of claim 28, wherein the agent is an antibody specific to an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 53-79 of SEQ ID NO:2, amino acids 53-79 of SEQ ID NO:4,

amino acids 54-80 of SEQ ID NO:5, amino acids 61-87 of SEQ ID NO:7, amino acids 61-87 of SEQ ID NO:9, amino acids 58-84 of SEQ ID NO:10.

30. The method of claim 16, wherein the agent is a dominant negative synaptotagmin I or II.

31. A method for identifying an agent that can block binding between BoNT/B and synaptotagmin I or II, the method comprising the steps of:

measuring binding between BoNT/B and a polypeptide in the presence of a test agent wherein the polypeptide comprises an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-79 of SEQ ID NO:2, amino acids 32-79 of SEQ ID NO:4, amino acids 33-80 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10, with the proviso that a polypeptide comprising a full length synaptotagmin I or II is excluded; and

comparing the binding to that of a control measured under the same conditions but in the absence of the test agent, wherein a lower than control binding indicates that the agent can block binding between BoNT/B and synaptotagmin I or II.

32. The method of claim 31, wherein the polypeptide consists of an amino acid sequence selected from the group consisting of amino acids 32-79 of SEQ ID NO:2, amino acids 32-79 of SEQ ID NO:4, amino acids 33-80 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

33. The method of claim 31, wherein all three steps are performed *in vitro*.

34. The method of claim 31, wherein the polypeptide is provided on a cell surface and the cell is exposed to the test agent.

35. The method of claim 34, wherein the binding between BoNT/B and the polypeptide is measured indirectly by monitoring the entry of BoNT/B into the cell.

36. A method for identifying an agent that can bind to a BoNT/B binding domain of synaptotagmin I or II, the method comprising the steps of:

exposing a polypeptide to a test agent wherein the polypeptide comprises an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10, with the proviso that a polypeptide comprising a full length synaptotagmin I or II is excluded; and

determining whether the agent binds to the polypeptide.

37. The method of claim 36, wherein the polypeptide consists of an amino acid sequence selected from the group consisting of amino acids 32-52 of SEQ ID NO:2, amino acids 33-53 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10.

38. The method of claim 36, wherein all three steps are carried out *in vitro*.

39. The method of claim 36, where the polypeptide is provided and exposed to a test agent in a cell.

40. A method for detecting BoNT/B or *Clostridium botulinum* comprising the steps of:

exposing a sample suspected of containing BoNT/B to a polypeptide that comprises an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 32-79 of SEQ ID NO:2, amino acids 32-79 of SEQ ID NO:4, amino acids 33-80 of SEQ ID NO:5, amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10, with the proviso that a polypeptide comprising a full length synaptotagmin I or II is excluded;

exposing the sample to a ganglioside wherein this step is optional when the polypeptide used comprises an amino acid sequence that is at least 70% identical to an amino acid sequence selected from the group consisting of amino acids 40-60 of SEQ ID NO:7, amino acids 40-60 of SEQ ID NO:9, and amino acids 37-57 of SEQ ID NO:10; and

detecting binding of the polypeptide to BoNT/B.